Evidences for Kaposi's sarcoma Originating from Mesenchymal Stem Cells through Mesenchymal-to-endothelial Transition

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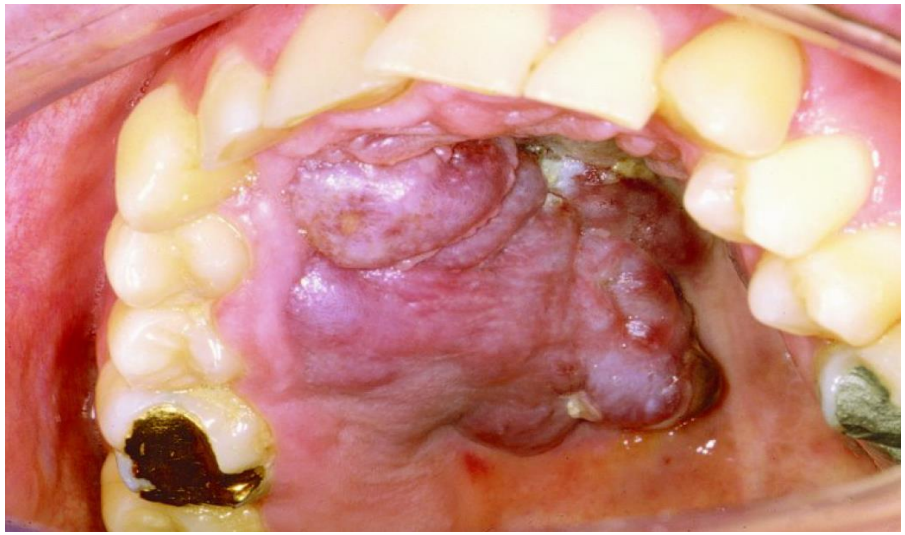
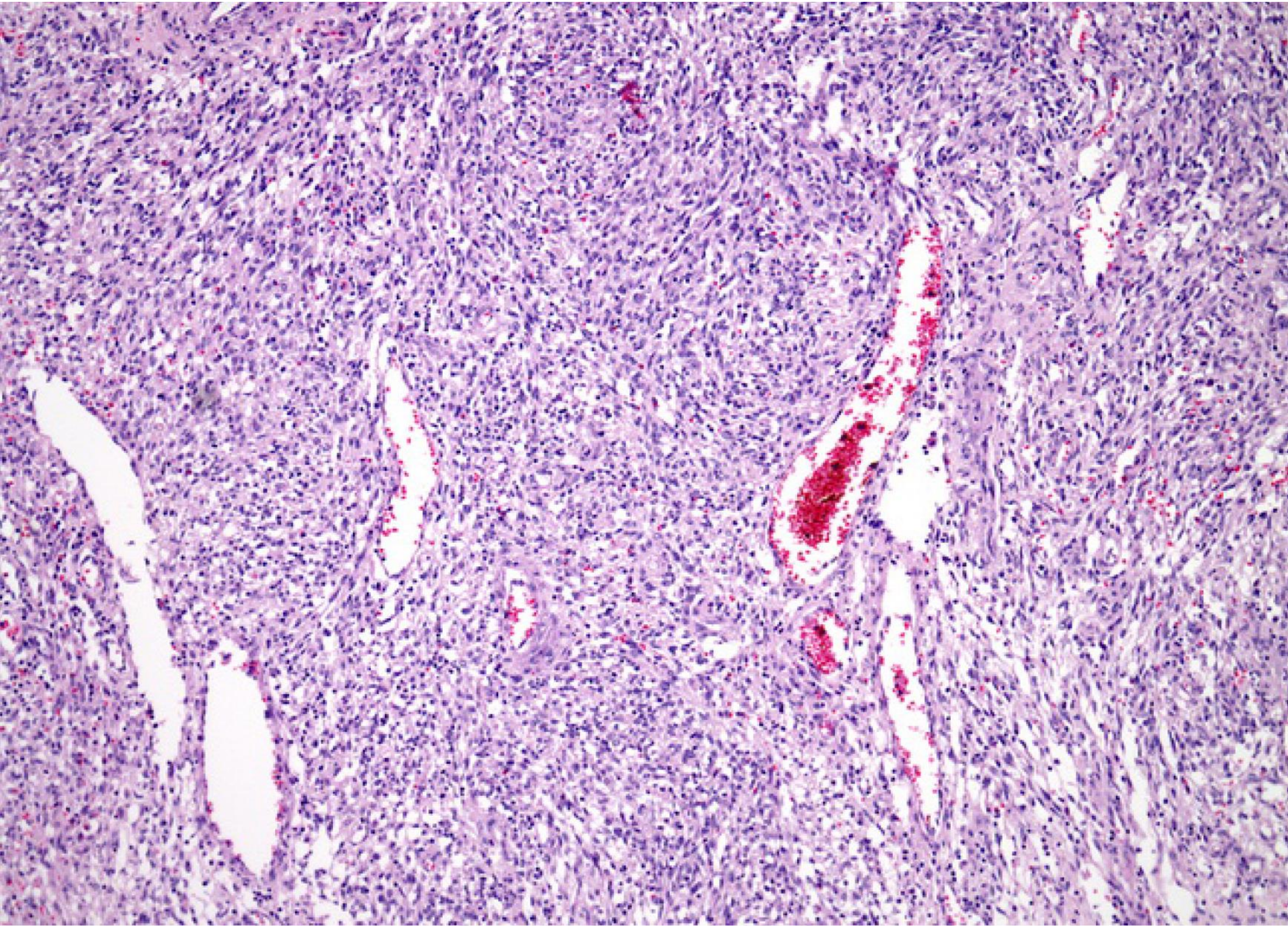
Oral Transmission of KSHV and AIDS-KS

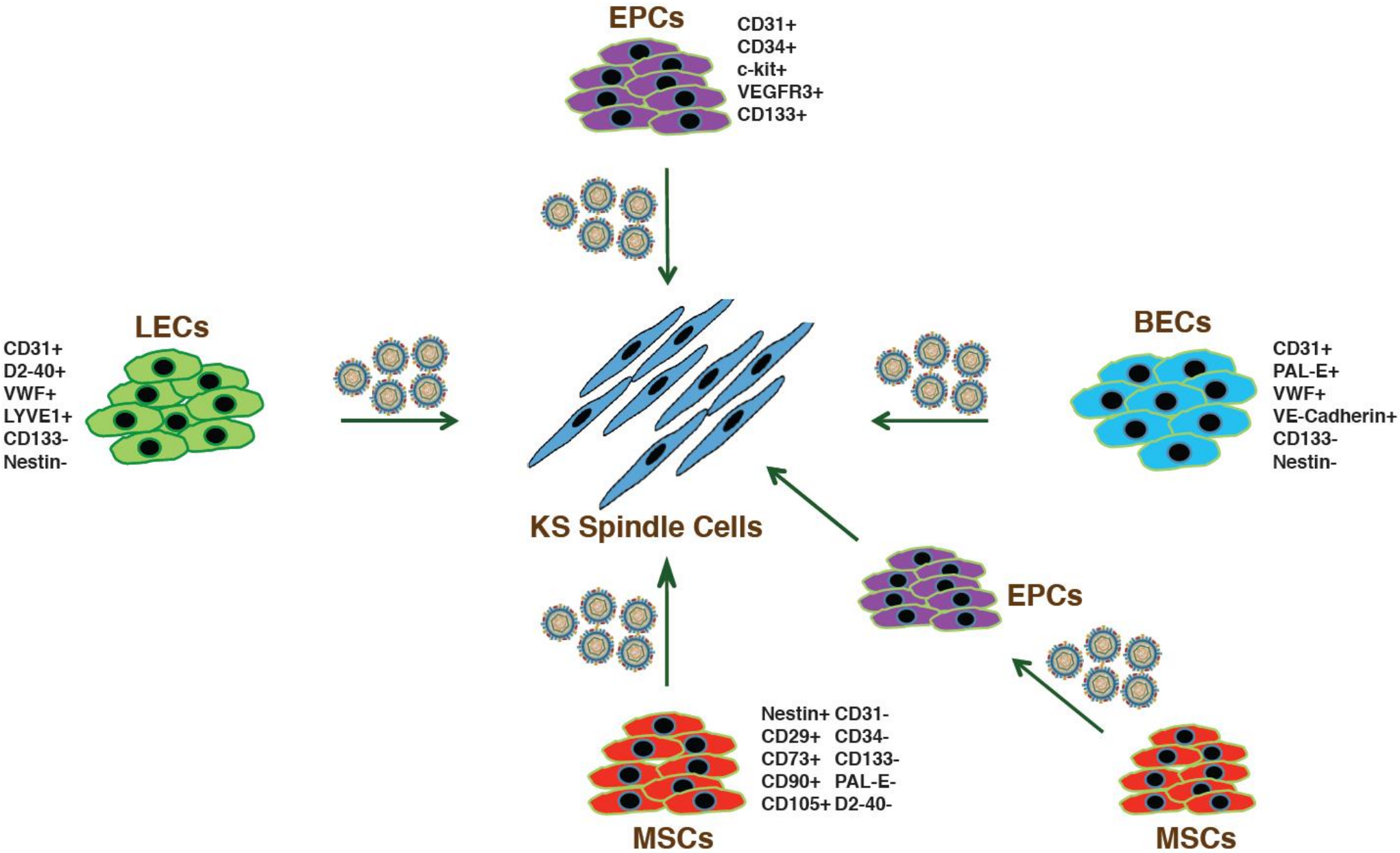
- KSHV is mainly shed in saliva of infected individuals and oral exposure to infectious saliva is the major risk factor for the acquisition of KSHV. Thus, oral transmission is the major route of KSHV infection.
- In AIDS-KS patients, oral cavity is often the first site of KS and the most common intraoral KS sites are palate or gingiva. Oral KS appears to be more aggressive and malignant.



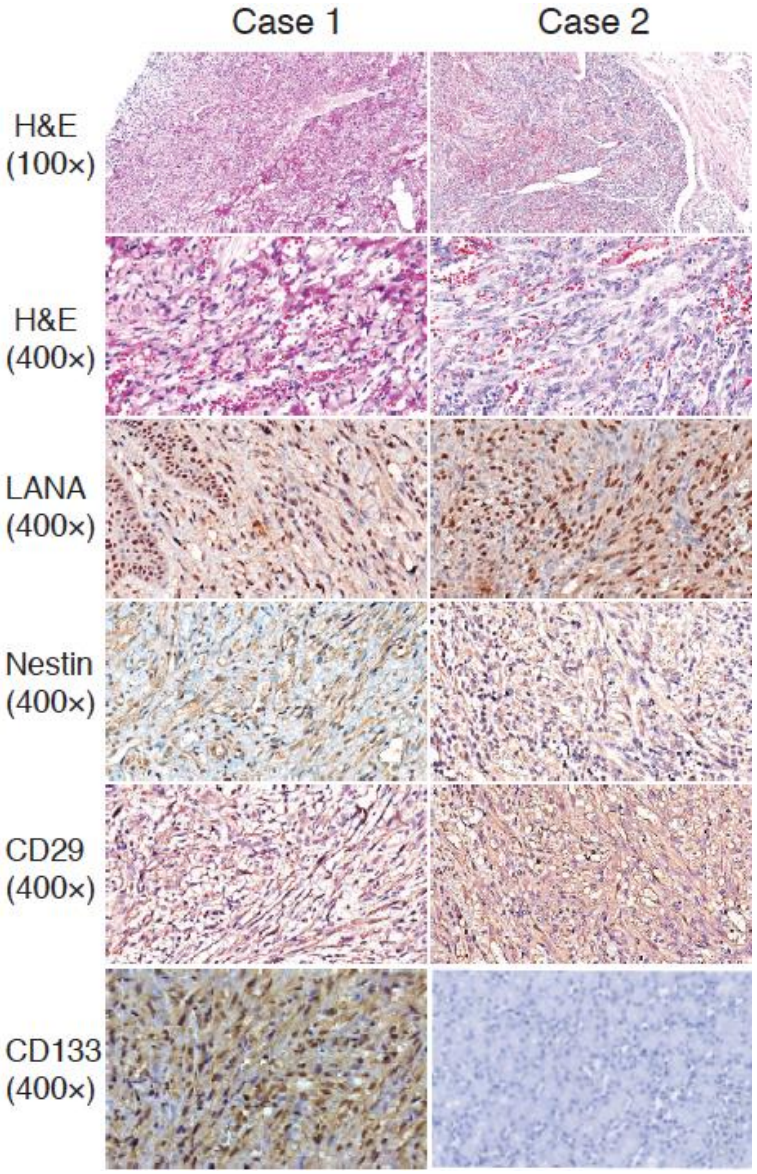
Questions: What is the primary target cell for KSHV infection in oral cavity? What is the cellular origin of KS spindle cells?



The Current Models for Origin of KS Spindle Cells

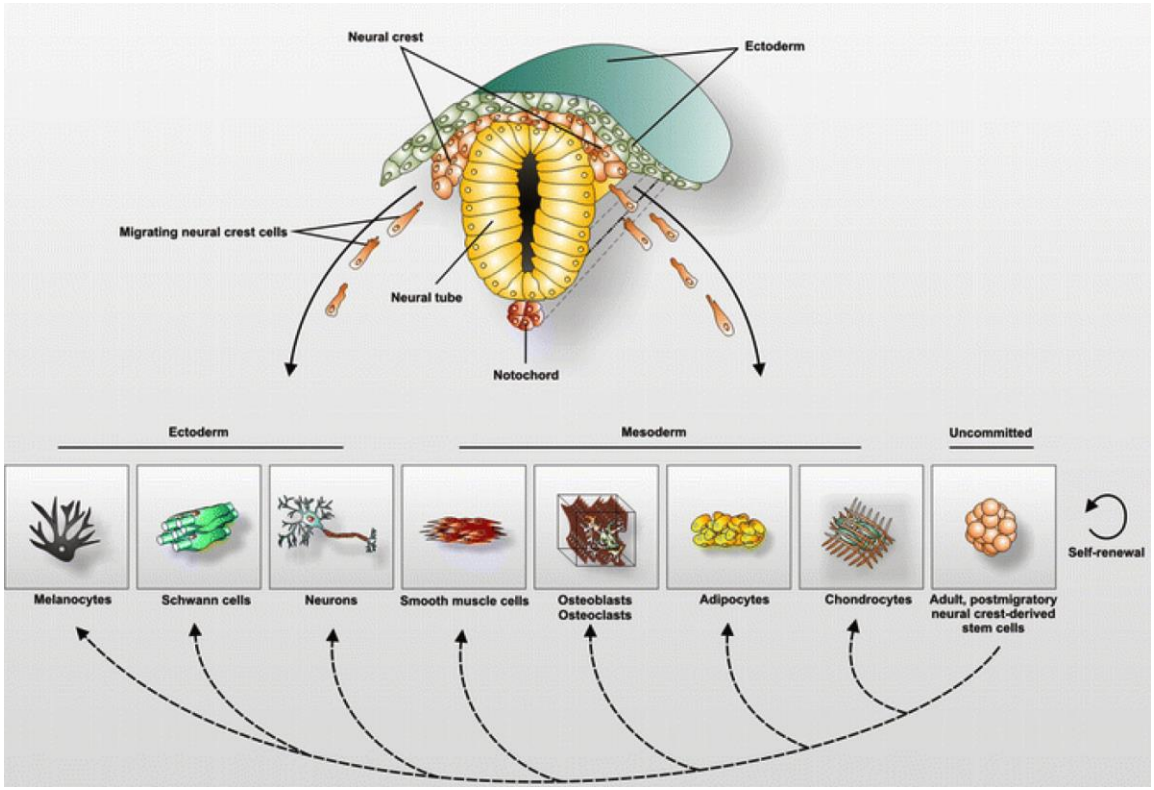


KS Lesions Express Neuroectodermal Stem Cell Marker (Nestin)

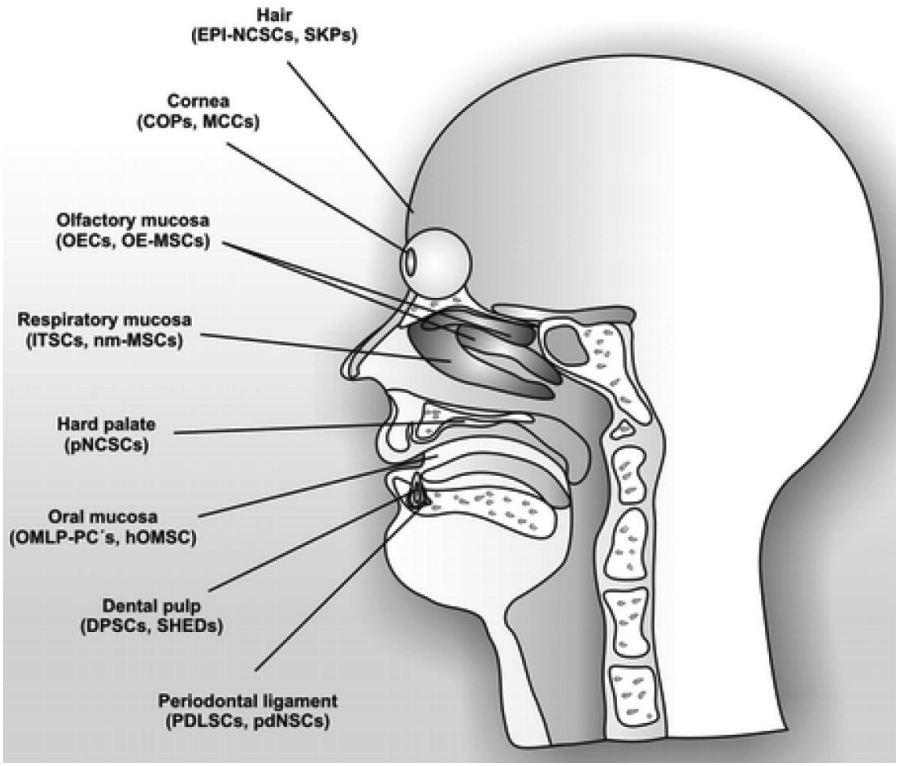


Case	KS Type	Age	Tumor Location	LANA	Nestin	CD29	CD34	CD133
1	AIDS-KS	31	Palate	+	+	+	+	+
2	AIDS-KS	30	Right Palm	+	+	+	+	-
3	AIDS-KS	24	Rectum	+	+	+	+	-
4	AIDS-KS	26	Cervical Lymph Node	+	+	+	+	+
5	AIDS-KS	61	Facial Skin	+	+	+	+	+

Expression of Nestin in KS Suggest A Lineage Connection Between MSCs and KS

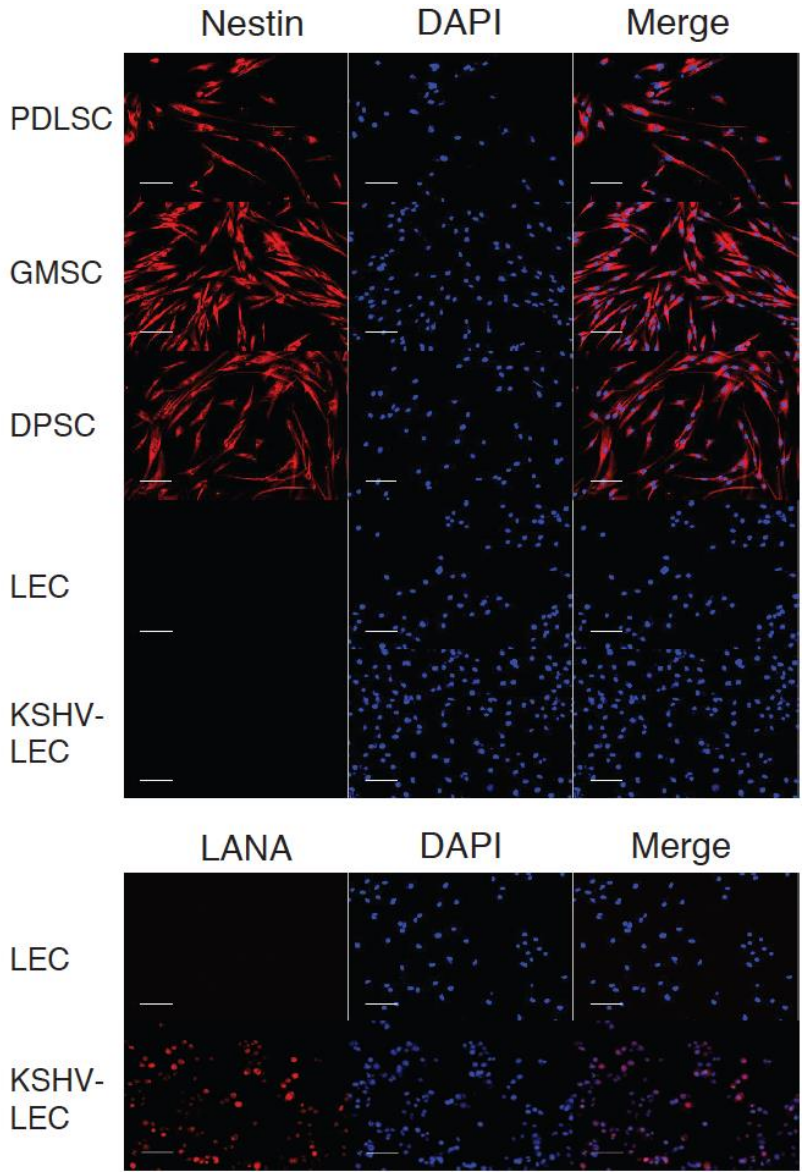


Neural Crest Cell Contributions to the Craniofacial Development



Neural Crest Cells migrate to form the craniofacial mesenchyme that differentiates into various cranial ganglia and craniofacial cartilages and bones.

Expression of Nestin in KS Suggest A Lineage Connection Between MSCs and KS



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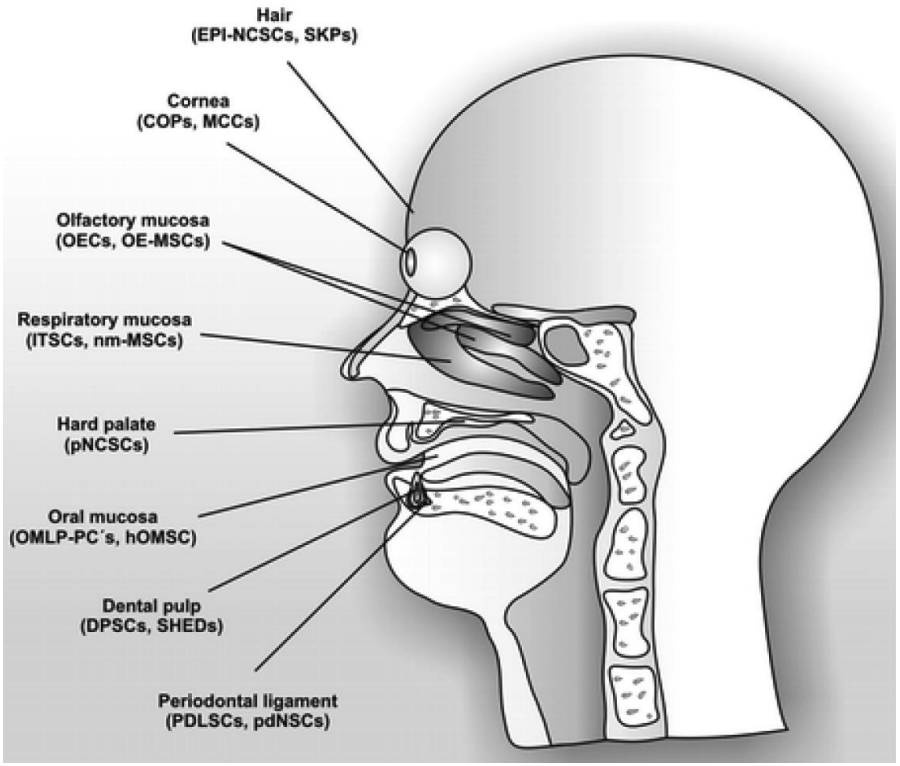
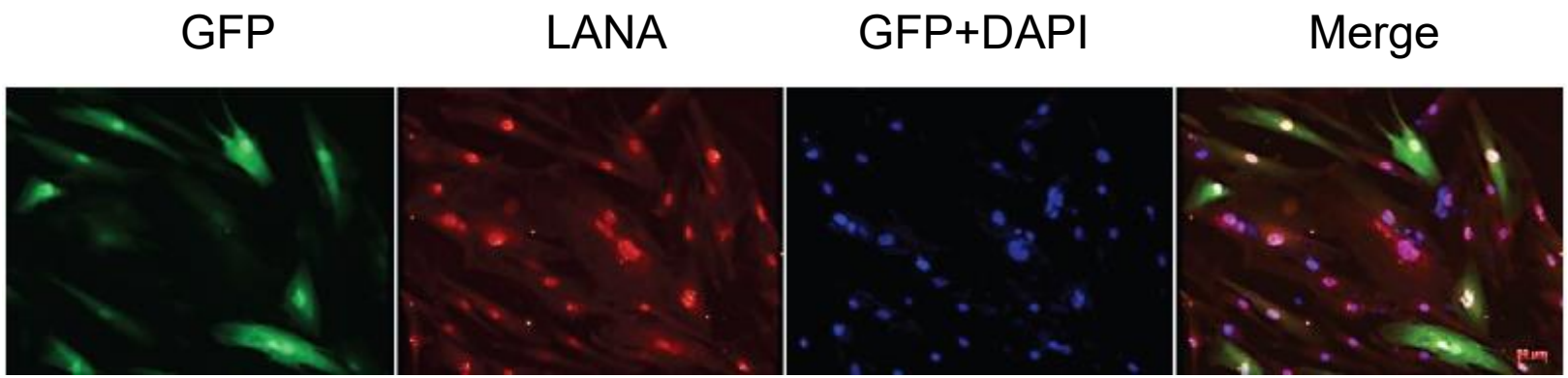
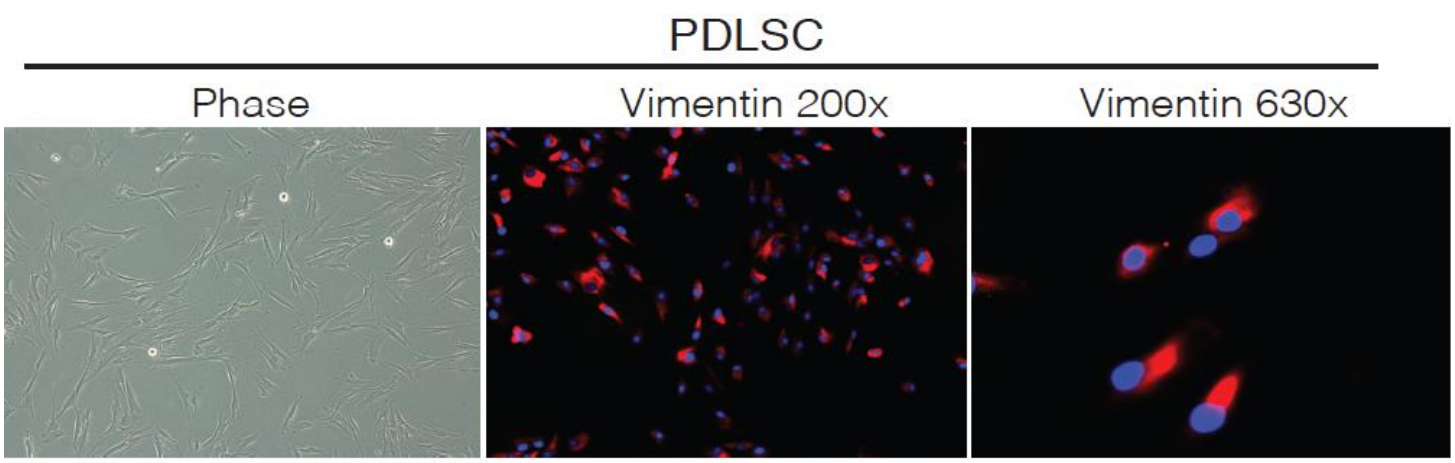


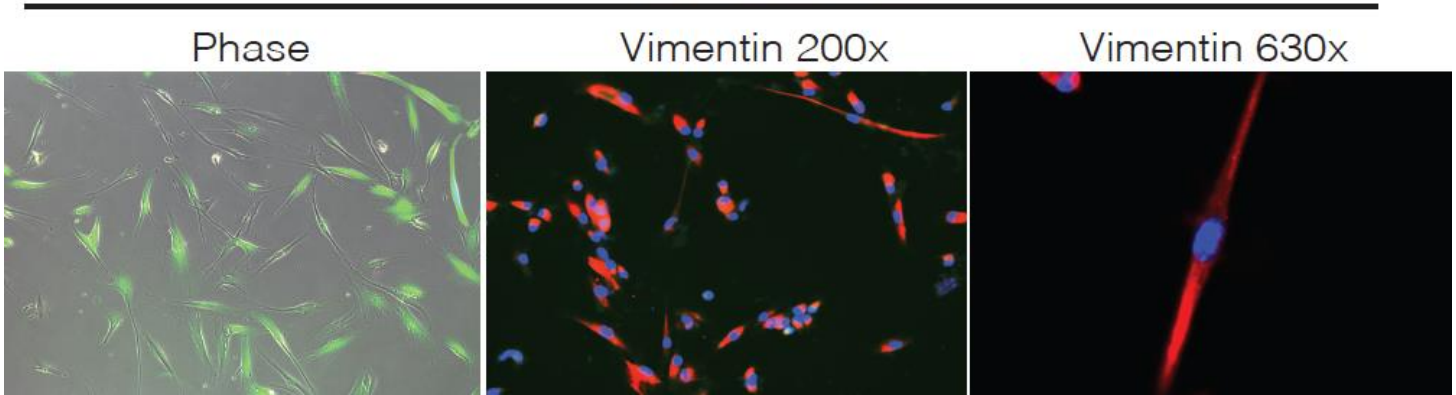
- *The expression of Neuroectodermal stem cell marker (Nestin) and oral MSC marker CD29 in KS spindle cells provides evidence that KS may originate from oral MSCs.*

- *KSHV infection of oral MSCs may result in a mesenchymal-to-endothelial (MEndT) transition, which leads to sarcomagensis of Kaposi's sarcoma (KS).*

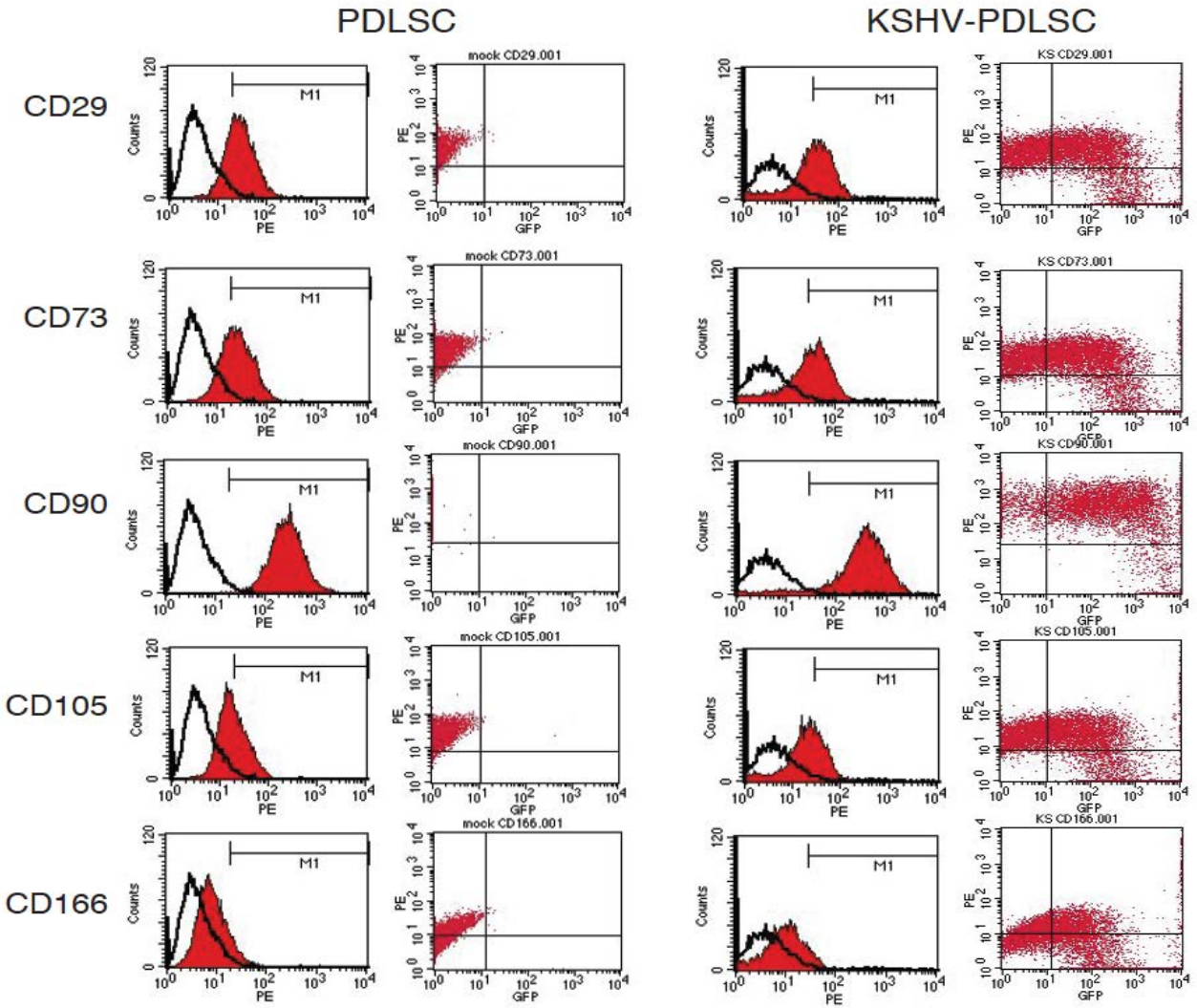
Oral MSCs Are Susceptible to KSHV Infection And Undergo Morphological Changes





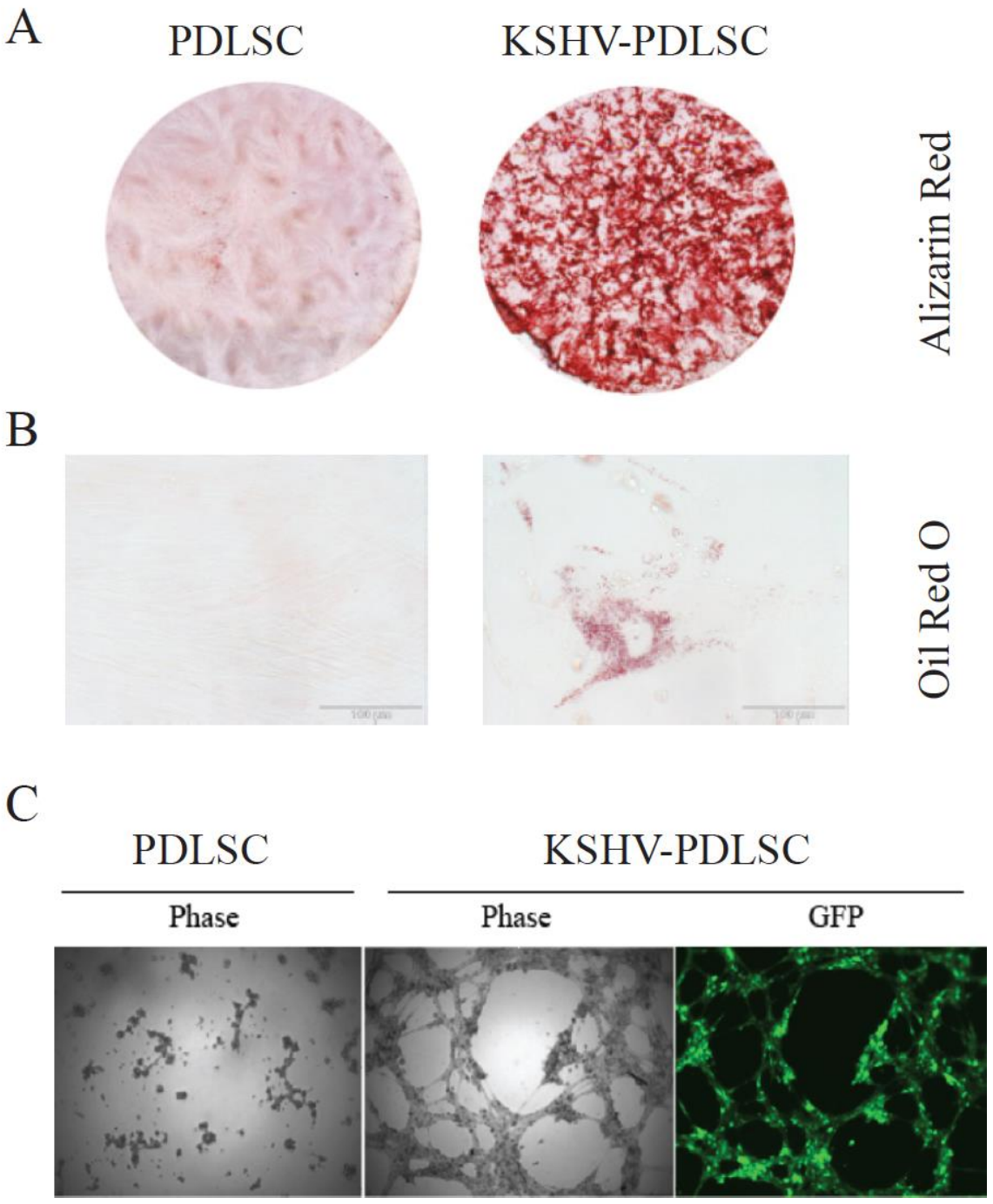


Surface Markers of Oral MSC Infected with KSHV

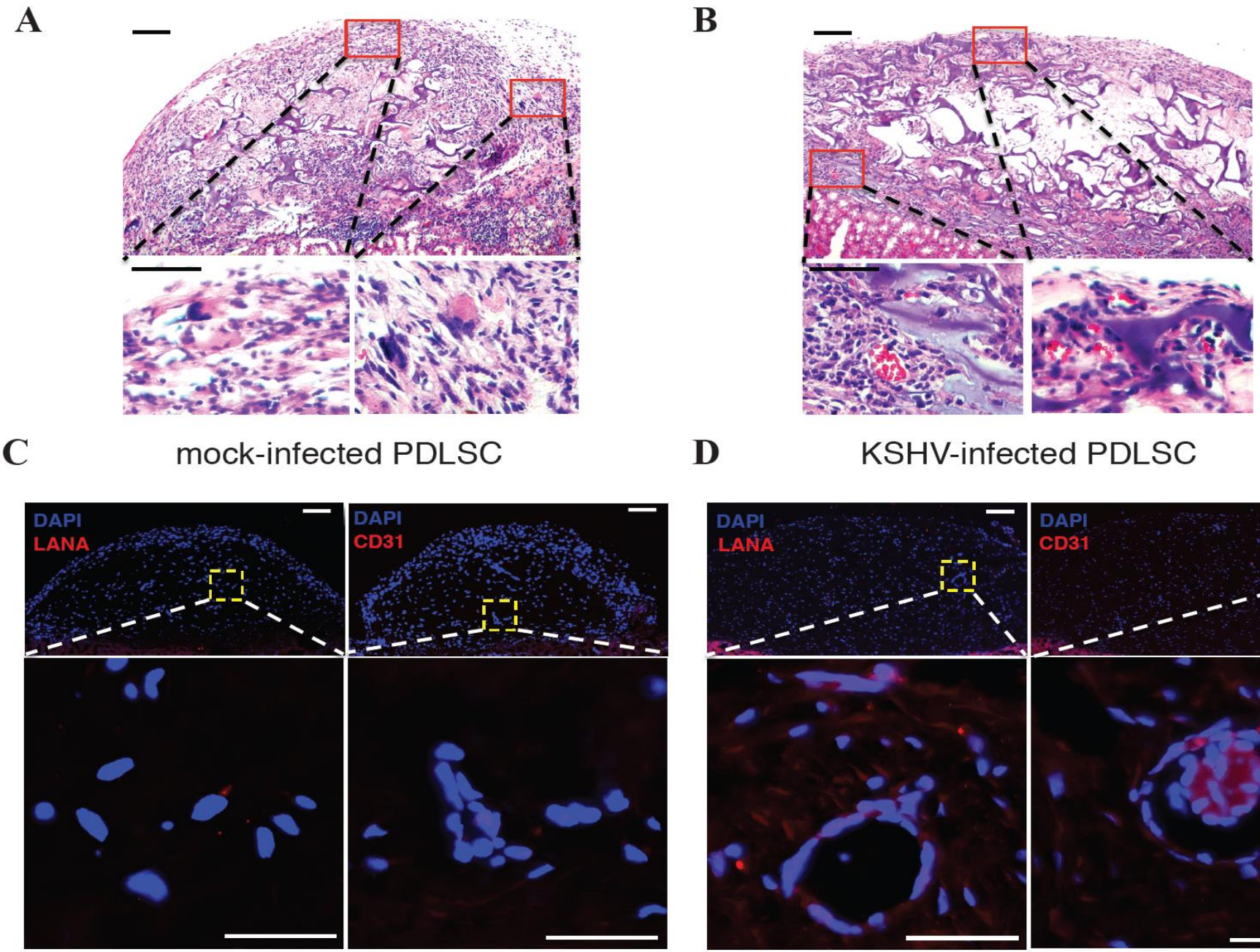


	CD31	CD34	CD44	CD90	CD105	CD166
PDLSC	9.5%	1.4%	98.8%	99.5%	98%	98.6%
KSHV-PDLSC	46.5%	18.1%	95.9%	89.4%	90.7%	85.7%

KSHV Infection Enhances Osteogenic, Adipogenic and Endothelial Differentiation



Kidney Capsule Implantation of KSHV-infected MSCs



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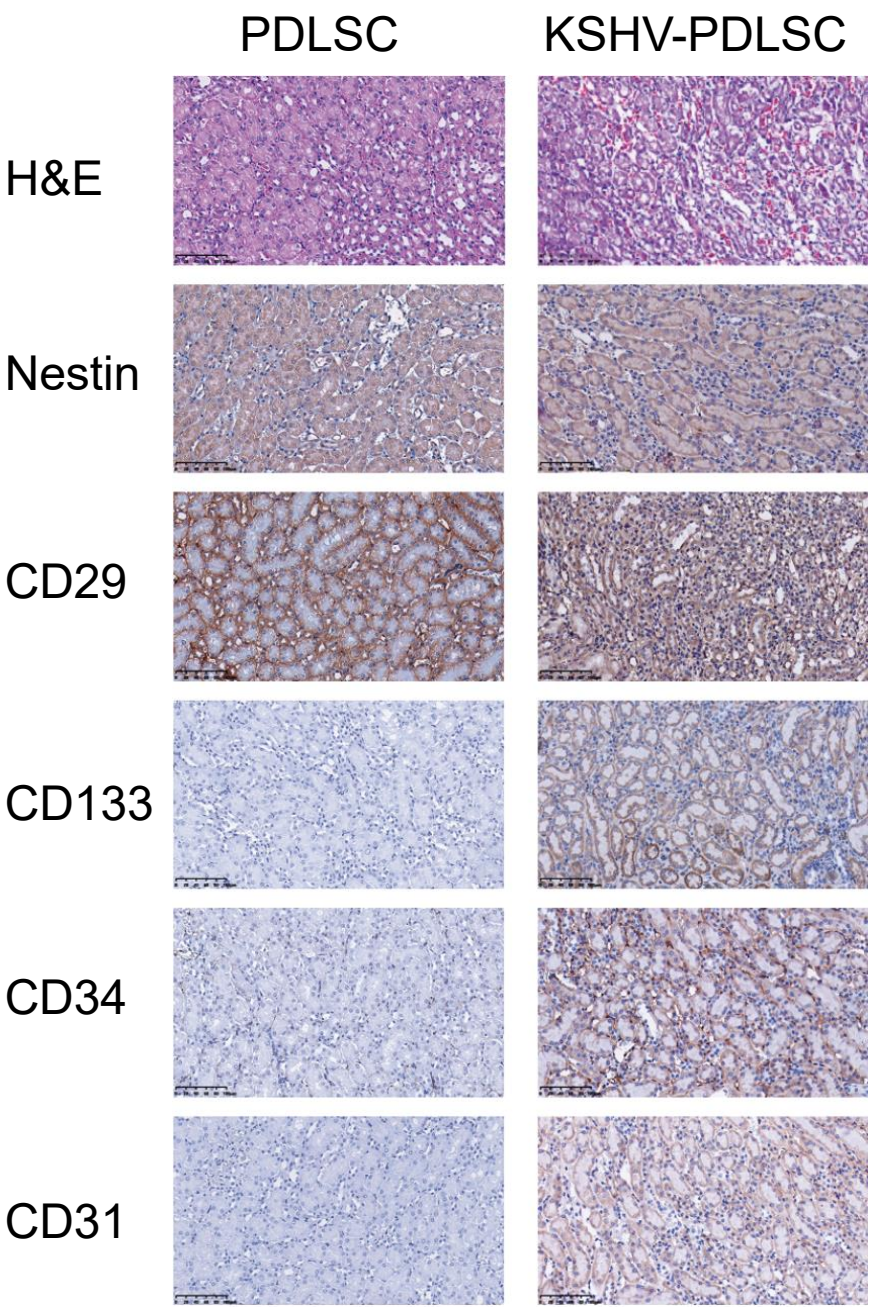
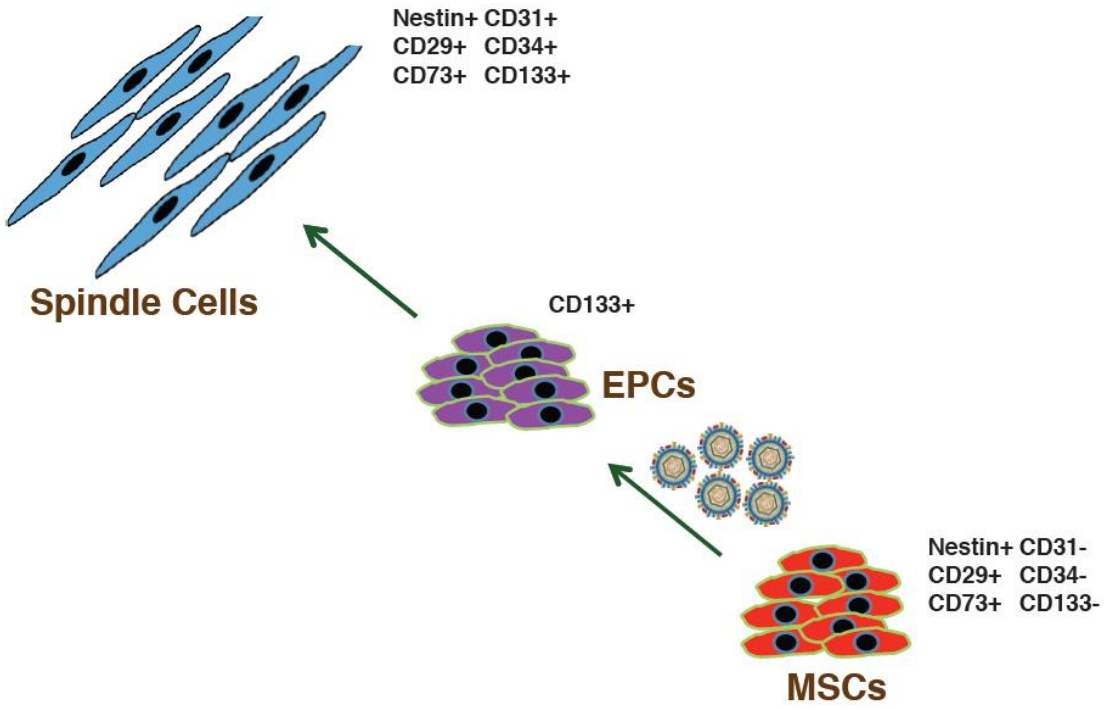
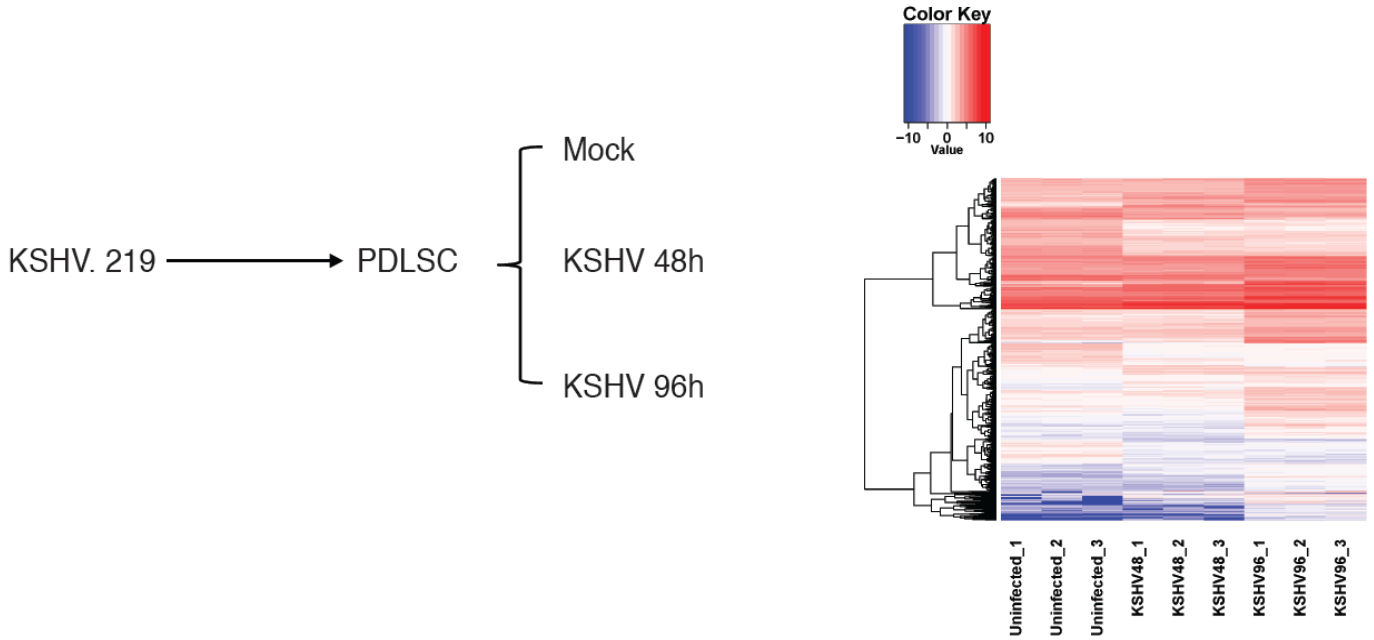


	Nestin	CD29	CD133	CD34	CD31	LANA
Mock-PDLSC	+	+	-	-	-	-
KSHV-PDLSC	+	+	+	+	+	+



RNA-seq Analysis for Gene Expression Profiles of MSCs upon KSHV Infection

A



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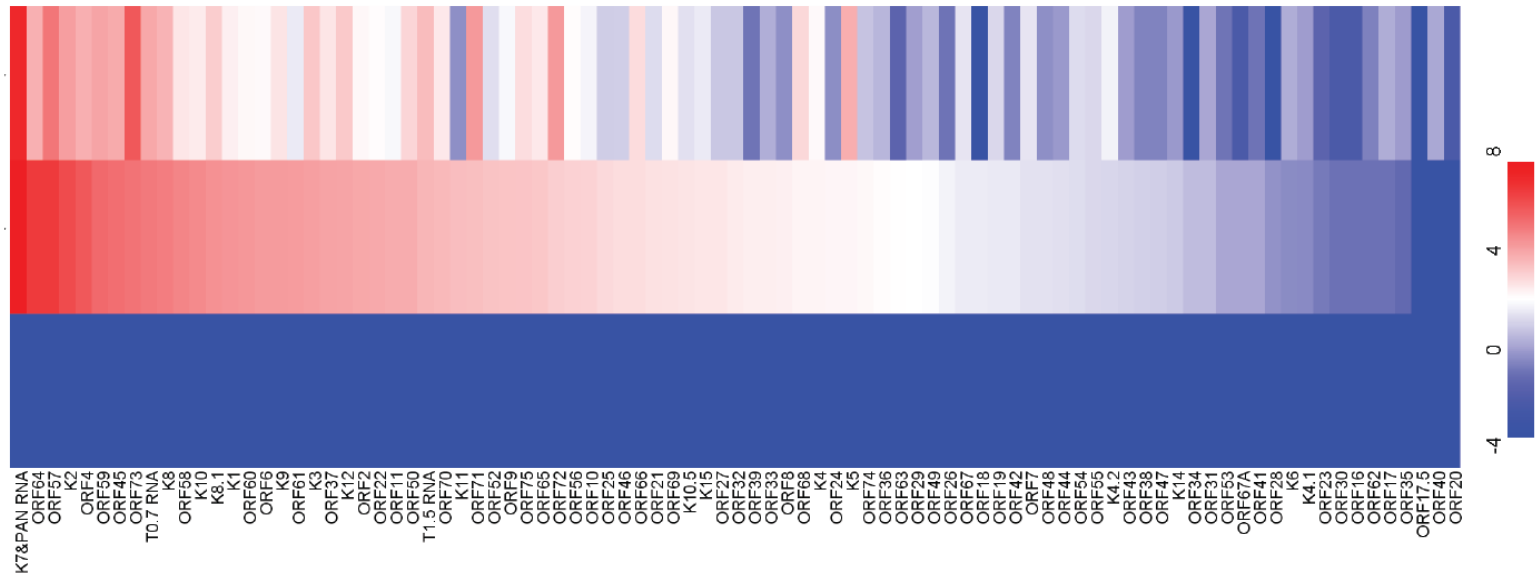
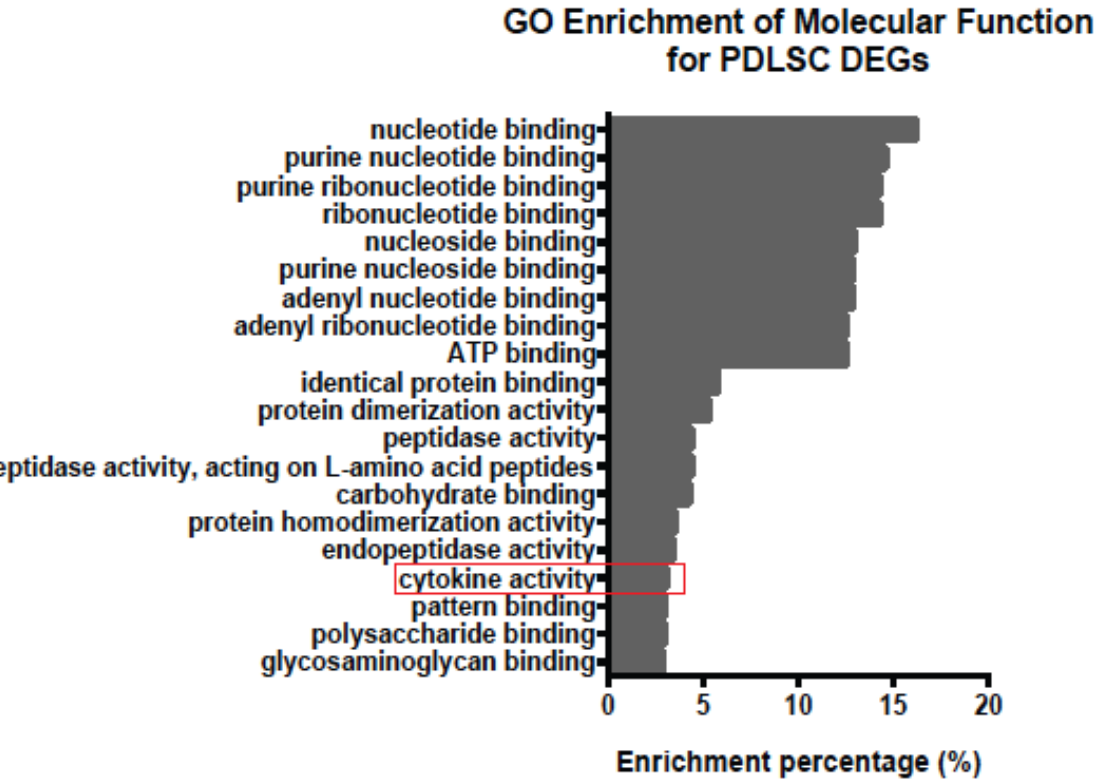
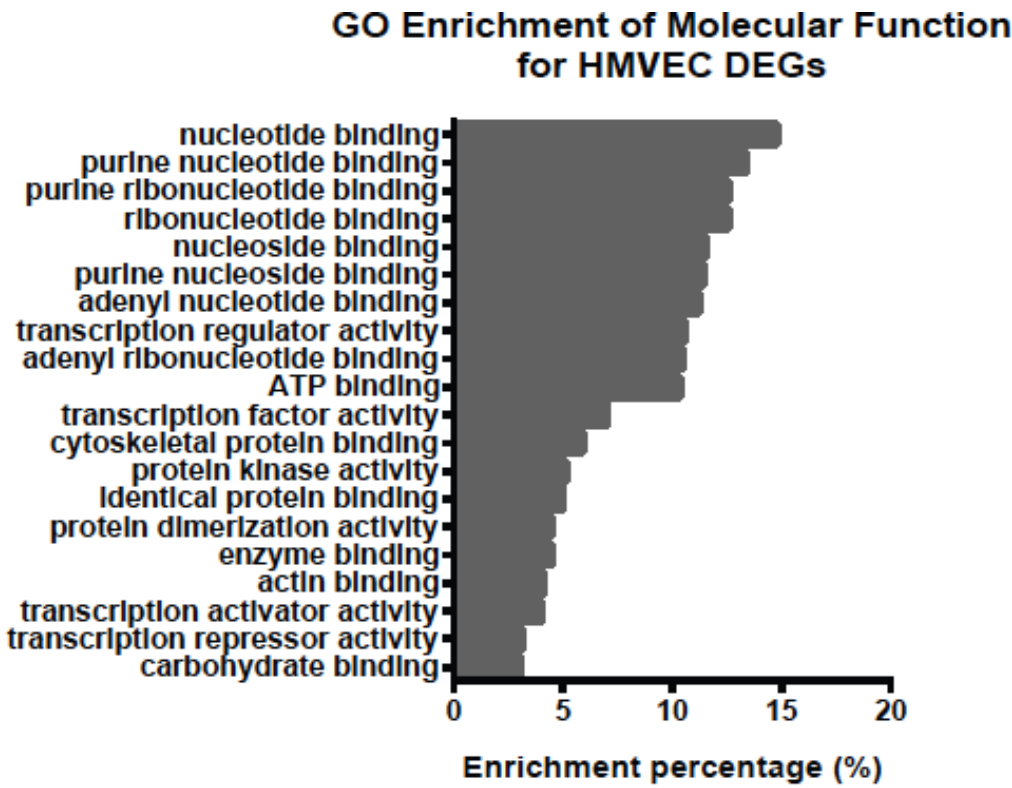


KSHV-induced Differentially Expressed Genes (DEGs) and Their Gene Ontology

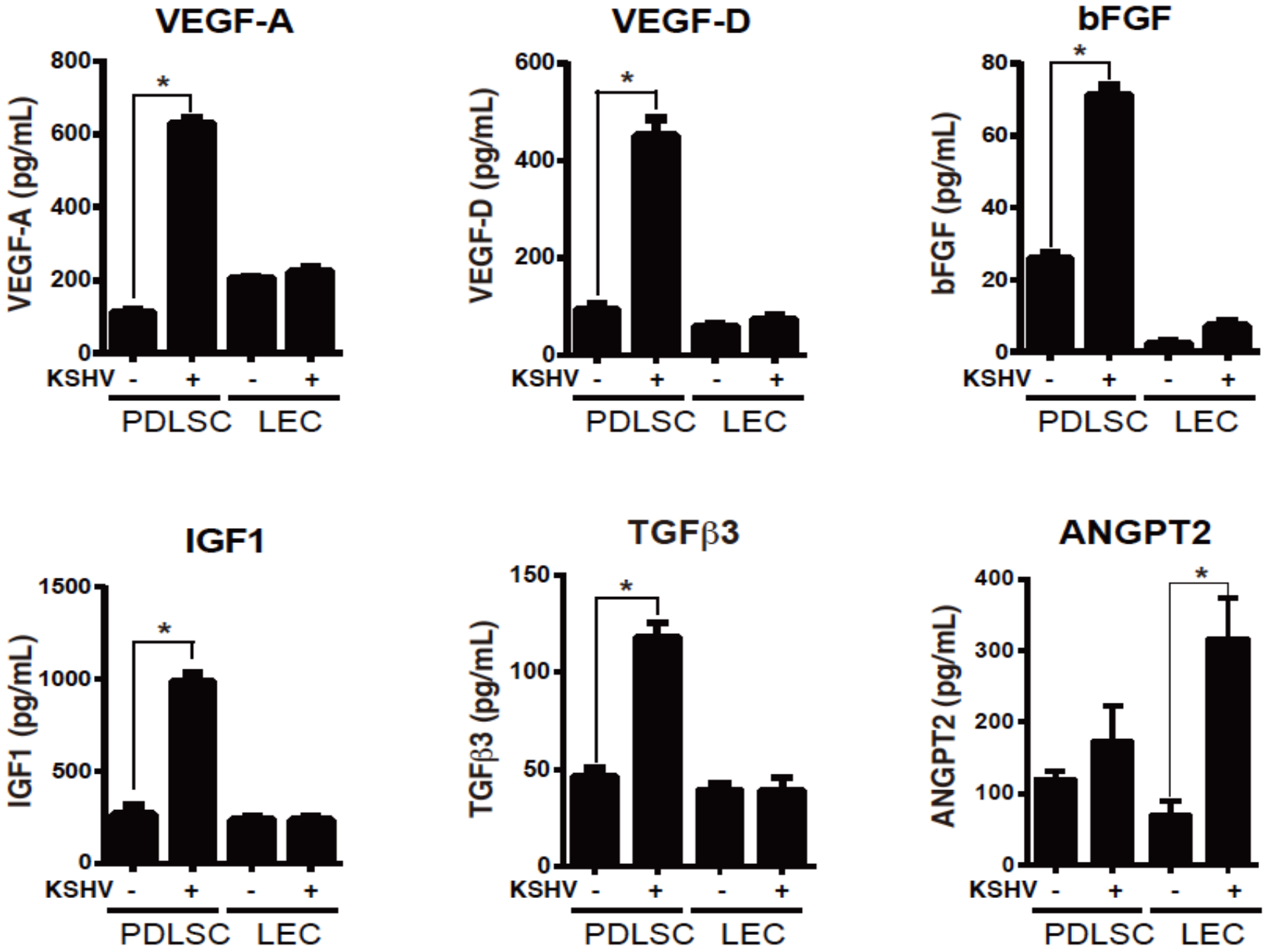




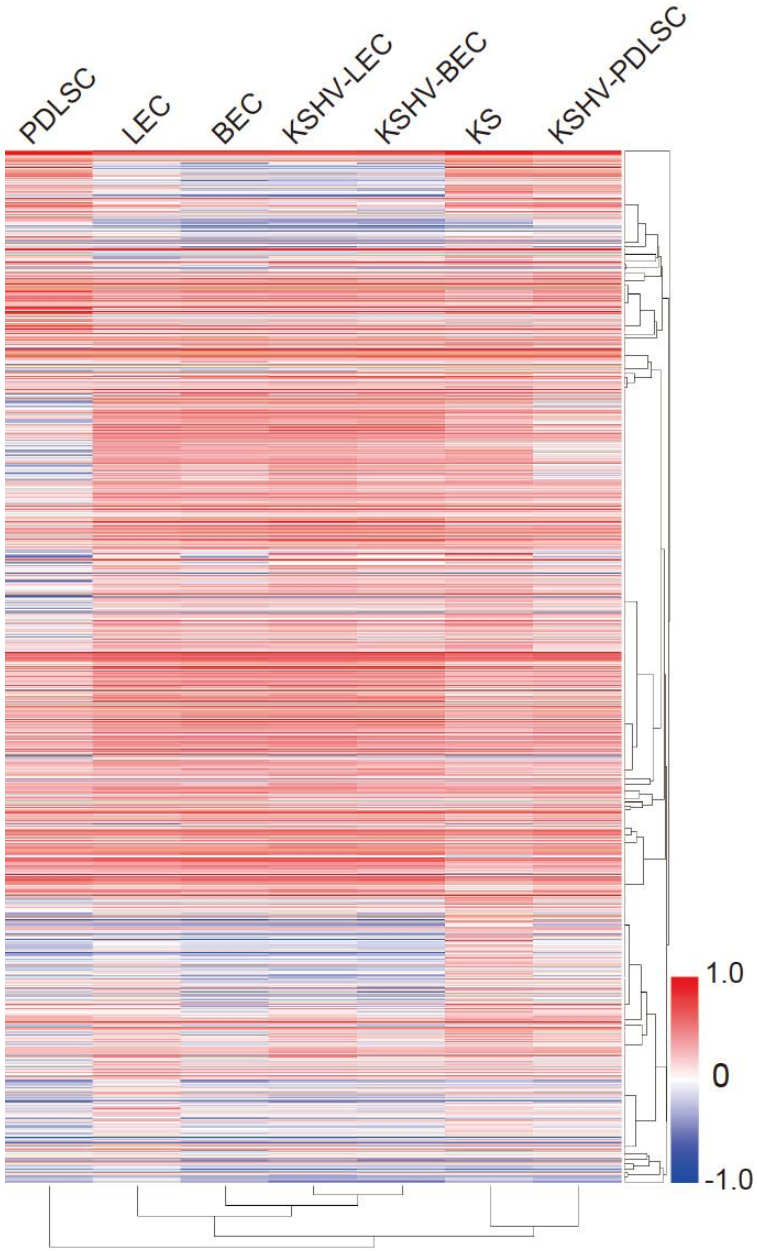
Cytokine Network in KSHV-infected PDLSCs

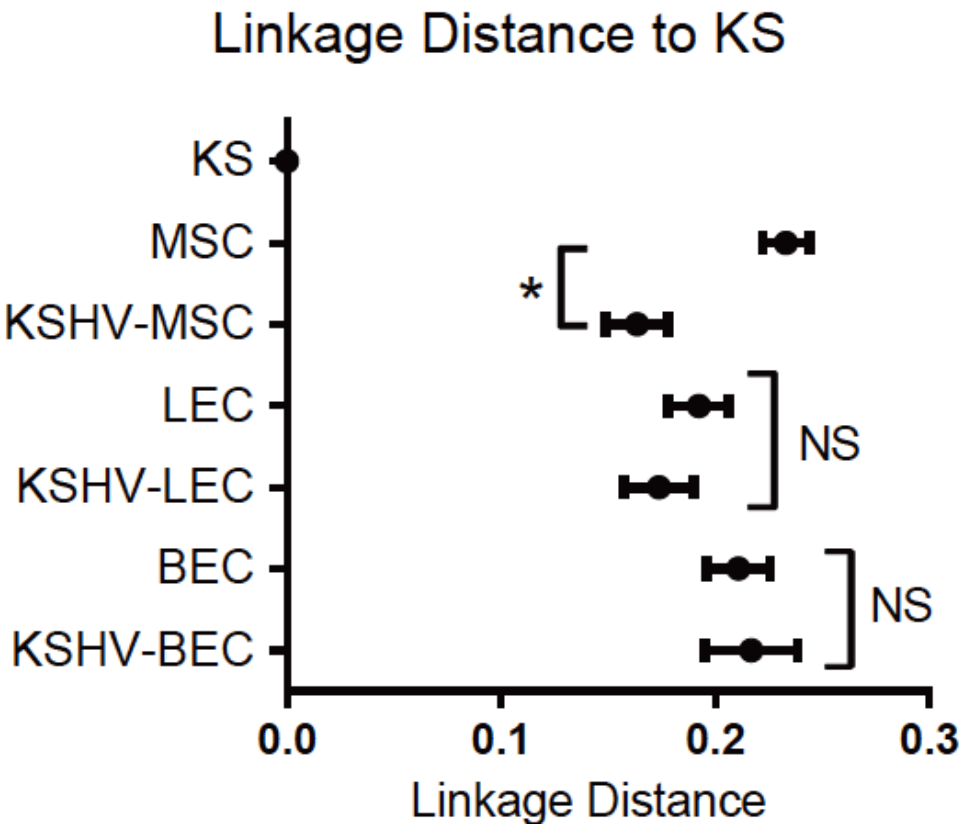
Function cluster	KSHV-PDLSC vs. PDLSC				KSHV-HDMEC vs. HDMEC				KSHV-HMVEC vs. HMVEC			
	Cytokine	Change	Receptor	Change	Cytokine	Change	Receptor	Change	Cytokine	Change	Receptor	Change
Chemotaxis	CCL2	+	CCR1	+	CCL14	+			CCL14	+		
	CCL5	+			CCL15	+			CCL15	+		
	CCL7	+	CCR3	+	CCL23	+			CXCL5	+		
	CCL8	+			CXCL13	-	CXCR5	+	CXCL10	+		
	CCL13	+			CXCL18	-	CXCR6	+	CXCL11	+		
	CXCL1	+							CXCL12	-	CXCR4	+
	CXCL3	+							CXCL16	+		
	CXCL5	+										
	CXCL6	+										
	CXCL10	+										
	CXCL11	-	CXCR7	+								
	CXCL12	-										
	CXCL16	+										
Endothelial development & angiogenesis	ANGPT4	+			ANGPT2	+			ANGPT2	+		
	ANGPTL1	+			LEP	-	LEPR	+	ANGPTL2	+		
	ANGPTL2	+			EDN1	-	EDNRB	+				
	ANGPTL4	+			FGF12	+						
	ANGPTL5	+			FGF13	+						
	EDN1	-	EDNRA	+								
			EDNRB	+								
	FGF2(bFGF)	+										
	FGF7	+										
	FIGF(VEGFD)	+										
	PGF	+										
	VEGFC	+	VEGFR3	+								
Inflammation	IL1α	+			IL1α	-	IL1R1	+	IL6	+		
	IL1β	+			IL1β	-			TNFSF4	+		
	IL6	+			IL3	-	IL3RA	+	TNFSF18	+		
	IL10	-	IL10R	+	IL5	+						
	TNFSF4	+			TNFα	+						
	TNFSF10	+			TNFSF10	+						
	TNFSF13B	+										
	TNFSF15	+										
Growth & differentiation	TGFβ3	+	TGFβR2	+	TGFα	+			PGF	+		
	OSM	+										
	IGF1	+										
	TSP1	-	CD36	+								
	INHBA	+										
	INHBB	+										

Cytokine Secretion in KSHV-infected MSCs and LECs

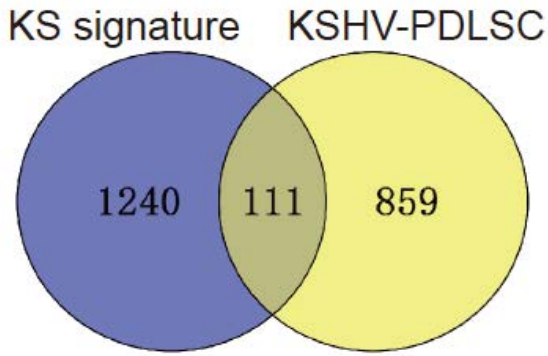


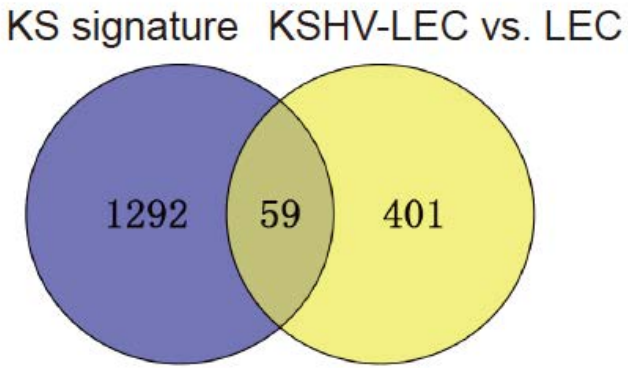
KSHV Signature Genes in Virus-infected MSCs

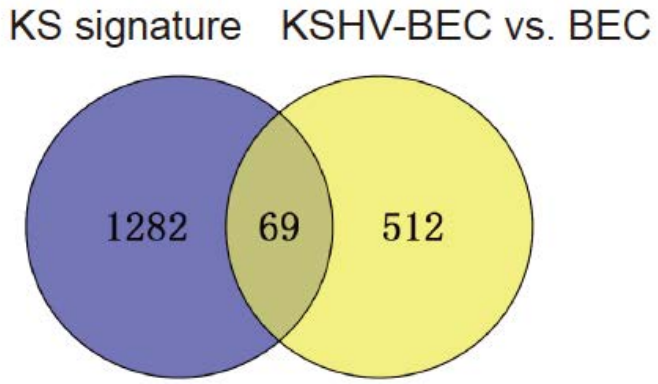


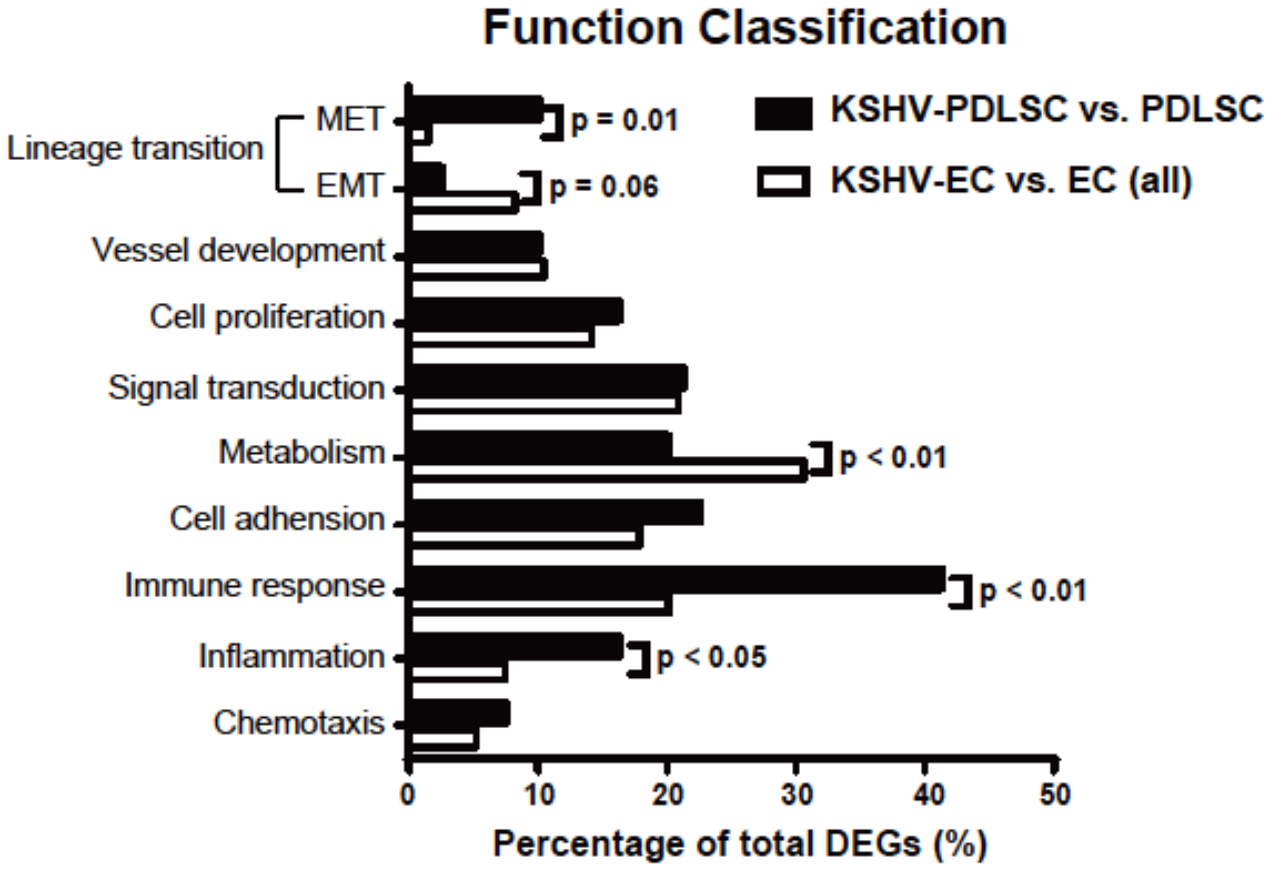


MEndT in KSHV-infected MSCs and EndMT in KSHV-infected Endothelial Cells







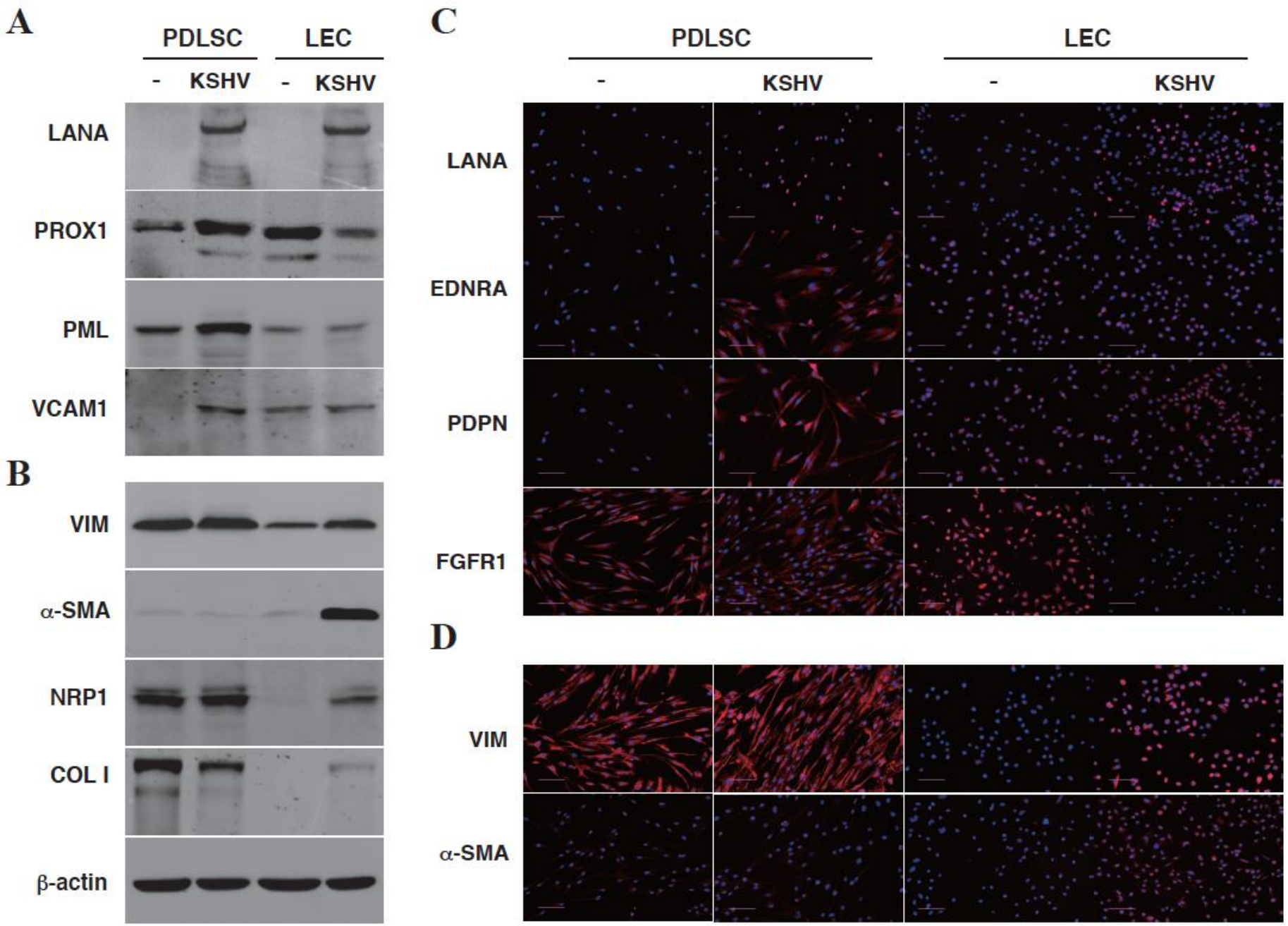


KSHV Signature Genes in Virus-infected MSCs

Common DEGs in KSHV-PDLSC vs. PDLSC and KS signature

Function Cluster		Gene symbol	KSHV-MSC vs. MSC	KS vs. skin	KSHV-HDMEC vs. HDMEC	KSHV-HMVEC vs. HMVEC	KSHV-LEC vs. LEC	KSHV-BEC vs. BEC
Mesenchymal to Endothelial Transition		BGN	↓↓	↑↑	–	↓	↓↓	↓
		DSP	↑↑	↓↓	–	↓	↓↓	–
		EDNRA	↑↑	↑↑	–	–	↓	–
		FGF2 (bFGF)	↑	↑↑	–	–	–	↓↓
		MMP11	↑	↑↑	–	–	↓	↑
		PDPN	↑↑	↑↑	↓↓	↑↑	↑	↑
		PROX1	↑↑	↑↑	↑	↓	↓↓	↑
		PGF	↑↑	↑↑	↓	↑	↓	–
		PML	↑↑	↑↑	–	–	↓	–
		TGFB3	↑↑	↑↑	–	–	–	–
		TGFBR2	↑↑	↑↑	–	–	↓	–
Vessel development		ANGPTL2	↑↑	↑↑	–	↑	↓	↑↑
		VCAM1	↑↑	↑↑	–	–	–	↓
		WARS	↑↑	↑↑	↑	–	↓	–
	Chemotaxis	CCL5	↑↑	↑↑	–	–	↑	↑↑
		CCL8	↑↑	↑↑	–	–	↑	–
		CXCL10	↑↑	↑↑	–	↑↑	–	–
		SHC3	↑↑	↓↓	–	–	–	–
		STAT1	↑↑	↑↑	↑↑	–	–	–
	Inflammation	APOL2	↑↑	↑↑		–	↓	–
		APOL3	↑↑	↑↑	–	–	–	↓
		CFH	↑↑	↑↑	–	–	–	–
		FN1	↑↑	↑↑	–	↓↓	↓↓	↓
		IFI16	↑↑	↑↑	–	↑↑	↑	–
		IL-1RN	↑↑	↓↓	–	–	–	–
		LY96	↑↑	↑↑	–	–	–	↑
		NT5E	↑↑	↑↑	↓	↑	↓	–
		PLA2G4C	↑↑	↑↑	–	–	–	–
	Other genes	FCGRT	↑↑	↑↑	–	–	–	–

MEndT in KSHV-infected MSCs and EndMT in KSHV-infected Endothelial Cells



Summary

- Oral mesenchymal stem cells can be efficiently infected by KSHV, suggesting these cells might be primary target cells for KSHV infection in oral cavity.
- AIDS-KS spindle cells express Neuroectodermal stem cell marker (Nestin) and oral MSC marker CD29, suggesting the oral/craniofacial MSC lineage of AIDS-associated KS.
- KSHV infection efficiently promotes oral MSC osteogenic, adipogenic and endothelial and angiogenic differentiation. KSHV-infected oral MSCs underwent endothelial differentiation in mouse kidney capsule, suggesting a mesenchymal-to-endothelial transition (MEndT).
- RNA-seq based gene expression profiling revealed that KSHV infection of oral MSCs results in up-regulation of a class of genes that are involved in MEndT and KS phenotypes, including production of a large number of chemokines/cytokines, growth factors and angiogenic factors, the characteristics of KS.
- Taken together, our results suggest that KSHV-infected oral MSCs could serve as progenitors of KS cells and MEndT driven by KSHV infection contributes to the development of KS.

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Wenjing Yu, Graduate Student

The Current Model for Origin of KS Spindle Cells

	CD31	D2-40	PAL-E	Nestin	CD29	CD133	LANA
LEC	+	+	+	-	-	-	+
BEC	+	-	+	-	-	-	+
EPC	+	-	-	-	-	+	+
MSC	-	-	-	+	+	-	+
MSC→EPC	-	-	-	+	+	+	+
MEndT	+	+	+	+	+	+	+
BEC⇋LEC	+	+	+	-	-	-	+

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